International Immunology, Vol. 4, No. 8, pp. 831 – 840

The expression of the mouse V_{preB}/λ_5 locus in transformed cell lines and tumors of the B lineage differentiation pathway

Akira Kudo[5], Philipp Thalmann, Nobuo Sakaguchi, Wendy F. Davidson[1], Jacalyn H. Pierce[2], John F. Kearney[3], Michael Reth[4], Antonius Rolink, and **Fritz Melchers**

Basel Institute for Immunology, Basel, Switzerland
[1]Laboratory of Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA
[2]Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA
[3]Division of Developmental and Clinical Immunology, Department of Microbiology and the Comprehensive Cancer Center, University of Alabama, Birmingham, AL 35294, USA
[4]Max-Planck-Institut für Immunologie, Freiburg, Germany

[5]Present address: Laboratory for Molecular Biology, Pharma Research Laboratories, Hoechst Japan, 3-2 Minamidai-1-Chome, Kawagoe City, Saitama 350, Japan

Key words: B lineage differentiation, V_{preB}, λ_5

Abstract

The expression of RNA transcripts from two pre B lymphocyte related genes, V_{preB} and λ_5, has been studied in a series of transformed cell lines which appear frozen at different states of B lineage differentiation, from early progenitors to surface Ig positive B cells. In the HAFTL-1 cell line, which arose from fetal liver by transformation with a retrovirus containing the H*ras* oncogene, Northern analysis of poly A$^+$ mRNA as well as *in situ* hybridization of RNA in single cells revealed that λ_5 and V_{preB} are already expressed at the progenitor stage and increase in expression as the progenitors differentiate to precursor (preB) cells, or are turned off as the progenitors differentiate to myeloid cells. Continued rearrangements of Ig genes in pre B cell lines leading to Ig expression on the surface of NFS-5 pre B cells do not influence the continued expression of V_{preB} and λ_5. Surface Ig-positive B lineage cell lines also express the pre B-related genes. Both Ly1$^+$ as well as Ly1$^-$ pre B cells are V_{preB}- and λ_5-positive. Lipopolysaccharide (LPS) stimulation of 70Z/3 pre B cells does not turn off λ_5 expression. It therefore appears that, at least in transformed cell lines, the expression of V_{preB} and λ_5 is not directly regulated by the expression of μH, κL, or λL chains, LPS reactivity, or the Ly1 surface antigen. Fusion of plasmacytoma cells with normal pre B cells to generate pre B hybridomas leads to down-regulation of V_{preB}/λ_5 expression. These results suggest that different *trans*-acting factors in more mature cells might down-regulate the expression of V_{preB}/λ_5.

Introduction

The differentiation of the B lymphocyte lineage in the mouse from stem cells to mature, antigen-sensitive, surface Ig-positive B cells has been characterized by surface marker expression, in particular by the Ly1 antigen, as well as by the states of Ig H and L chain gene rearrangements and expression in transformed cell lines and normal cells of fetal liver and bone marrow, and also by sensitivities to polyclonal activators and cytokines of normal cells (1 – 6). Two closely linked genes V_{preB} and λ_5 encoding 0.85 and 1.2 kb mRNA molecules respectively (7 – 10) have been found to be selectively expressed in precursor (pre) B lymphocytes. To further specify the pattern of expression of these two genes this paper deals with the following objectives:

(i) When does transcription of V_{preB1} and λ_5 begin, as stem cells develop to progenitor (pro) B cells (11 – 13) and then to the

Correspondence to: A. Kudo

Transmitting editor: T. Watanabe

Received 3 February 1992, *accepted* 13 April 1992

various stages of pre B cells? To approach this problem we investigate the V_{preB}/λ_5 gene expression in a cell line, HAFTL-1, which was transformed by a retrovirus containing the H*ras* oncogene of Harvey sarcoma virus, and which shows properties of a novel lymphoid progenitor cell line (14). The cell line has been characterized in greater detail as a precursor cell committed to the Ly1$^+$ B cell differentiation pathway, which is Thy1$^-$, Mac-1$^-$, 8C5$^-$, Ly5(B220)$^-$, Ly6-2$^+$, Ly8$^+$, and Ly17$^+$ (15,16). It appears to be actively involved in the process of D_HJ_H rearrangements, since its DNA is partially in germline and partially in one predominant D_HJ_H rearranged state (17). From this cloned cell line, subclones which have characteristics of either macrophages or pre B cells were developed by continuous stimulation with lipopolysaccharide (LPS). The macrophage subclones analyzed in this paper express Mac-1 and Mac-2, produce lysozyme and unspecific esterase, and show the one predominant D_HJ_H rearrangement already seen in the precursor cell. On the other hand, three pre B subclones (clones 6, 7, and 14) studied in this paper express the ThB marker, and two of them (clones 7 and 14) show additional J_H rearrangements, indicating that, in contrast to the macrophage lines, the pre B lines continue to rearrange their H chain locus. These results collectively indicate that the HAFTL-1 cell line has the potential to differentiate into either the pre B or the myeloid cell lineage. We investigate here the expression of V_{preB1} and λ_5 at the different stages of differentiation of the HAFTL-1 cell line.

(ii) Does the transcription of Ig L chain genes, and their expression as proteins, lead to a down-regulation of V_{preB} and λ_5 transcription? The most mature cell line of the B lineage which, thus far, has been found to express the V_{preB}/λ_5 locus is the cell line from which, in fact, the λ_5 gene was originally isolated, i.e. the pre B lymphoma line 70Z/3 (7,18). All Ig-positive B cell lines (as well as all Ig-positive normal B cells) investigated so far were found not to express V_{preB}/λ_5 (7,8,10). This has suggested that the V_{preB}/λ_5 locus is turned off when H and L genes have been rearranged and when surface Ig appears on cells of the B lineage. If the effect of expressing L chain genes into proteins on the down-regulation of the V_{preB}/λ_5 expression were to be direct, one might expect that the subsequent expression of the κ or λ chain locus in pre B lymphoma cells originally expressing only the μ chain gene locus might then turn off V_{preB}/λ_5 expression. Here we test for expression of λ_5 in subclones expressing only the H chain locus, or the H chain and the λL chain locus of a continuously rearranging pre B lymphoma cell line, NFS-5 (19,20), and other intermediate cell lines from pre B to mature B.

(iii) What happens to V_{preB}/λ_5 gene expression when normal pre B cells are fused to a plasmacytoma? It has previously been found that normal antigen-sensitive, mature, Ig-positive B cells and Ig-secreting plasmablasts and plasma cells do not express these genes (7,8,10). in contrast to transformed cell lines with pre B phenotype which express V_{preB} and λ_5, hybrids between a plasmacytoma and normal pre B cells retain chromosomes from both cells. They should test whether expression of V_{preB}/λ_5 is dominant over non-expression or vice versa. The pre B hybridomas used in our studies were derived from fusions of normal pre B cells with the plasmacytoma X63 Ag8.653 (21) and contained H chain chromosomes in rearranged forms and L chain chromosomes in germline configuration (22,23).

(iv) Is the expression of V_{preB}/λ_5 restricted to one of the two lineages of B cell differentiation, i.e. to Ly1$^+$ or Ly1$^-$ cells (6)? The results of this paper, studied with transformed cell lines and hybridomas, give clues as to in which stages of normal B cell development the V_{preB}/λ_5 locus might be expressed.

Methods

Cell lines and tissue culture

The HAFTL-1 cell line was derived by *in vitro* transformation of NFS/N fetal liver cells with Harvey murine sarcoma virus as previously described (14,15). Subclones with either pre B cell or macrophage characteristics were established and characterized as described (16,17). Progenitor HAFTL-1 cells at early (HAFTL-1, p8) and at later stages (HAFTL-1, Sc-1(p); and HAFTL-1, Sc-2(p)) of cell culture passages (p) are compared with three pre B-type subclones (clones 6, 7, and 14), as well as three macrophage-type subclones (IG4, 2B7, and 3G4).

The Ly1$^+$ B lineage lymphomas NFS-1, NFS-5, and NFS-25 were generated by inoculation of newborn NFS/N mice with ectopic murine leukemia virus (CAS25M) (19). Hardy *et al.* (24) cloned a series of cell lines from the original NFS-5 line which were shown to have differentiated further along the pathway of Ig gene rearrangements. Thus, from an original $\mu^+\kappa^-$ NFS-5 line a $\mu^+\kappa^+$ cell line, then $\mu^+\kappa^+\lambda^+$ and finally a $\mu^+\lambda^+$ cell line was generated. We thank Drs R. Kleinfield and M. Weigert (Institute for Cancer Research, Fox Chase, Philadelphia, PA, USA) for a Northern blot containing mRNAs prepared from these four stages of NFS-5 development, used in the results shown in Fig. 4 (20).

The Ly1$^+$ pre B cell line ABLS-19, the Ly1$^-$ pre B cell line ABLS-8, and the Ly1$^+$ plasmacytoma line ABPC69 were established and characterized as described (15). The sources of the cell lines 70Z/3, A32-26, and 18-81 are described elsewhere (7). 38C-13 is a carcinogen-induced Ig-positive B lineage cell line (25) with some pre B characteristics (26) obtained from Dr J. Haimovich (Department of Human Microbiology, Tel Aviv University, Tel Aviv, Israel).

The Ig-non-producing plasmacytoma cell line X63.Ag8.653 has been described previously, and the pre B hybridomas ND4.4-1, JC2.2, IA2.2.2, and HA3.18 were derived from fusion of the Ag8.653 parent line with normal pre B cells (21 – 23).

The mature B cell line WEHI 279 (33) and the thymoma EL4 were gifts from Dr J. McKearn at our Institute (now at Dupont Glenolden Laboratories, Glenolden, PA, USA). The thymic lymphoma BW5147 was given to us by Dr Hyman (Salk Institute for Biological Studies, La Jolla, CA, USA). The macrophage lines P388D1 and WEHI 3 were obtained from Dr N. Iscove at our Institute (now at the Ontario Cancer Institute, Toronto, Canada). All cells were kept in Iscove's modified Dulbecco's medium (IMDM) containing 5×10^{-5} M 2-mercaptoethanol, kanamycin (Gibco), and serum-substituting concentrations of human transferrin, bovine serum albumin, and soybean lipids as described.

Rolink *et al.* have recently established stroma cell and IL-7-dependent normal pre B cell clones (37). PAB clone 5, one of the established pre B clones, was grown with serum substituted IMDM (27) on a semi-confluent layer ($1 - 3 \times 10^4$/ml) of PA6 stromal cells, which had been treated with 3000 rad of γ irradiation, in the presence of 100 – 200 U/ml Il-7.

A surface $\mu\kappa$ positive mouse B cell line, Ig6.11, was a kind

gift from Dr Takemori (34). The Ig6.11 cell line was established by subcloning an immature B cell clone 46-6 that had been cultured at a non-permissive temperature. The 46-6 clone was obtained by transforming BALB/c bone marrow cell with a *ts* mutant of Abelson murine leukemia virus.

RNA preparation and analysis

Preparation of cytoplasmic RNA for RNA dot or slot blot analysis or Northern blot analysis, agarose gel electrophoresis, and transfer to nitrocellulose filters was done by standard procedures.

Analysis

The 300 bp *Hin*dIII – *Stu*I DNA fragment of pZ-183-1a was used as a λ_5 specific probe (9). V_{preB1} was the *Eco*RI – *Acc*I fragment of cDNA clone pZ121 (10). The *Pst*I-digested insert fragments of pABμ (as μ probe), the 2 1 kb *Pst*I fragment of pAL 41 plasmid (as β-actin probe), the *Hin*dIII – *Bam*HI fragment of pECK (as κL chain probe), and the 420 bp *Xho*I – *Pst*I fragment olf pA8-1-1 (as λ light chain probe) were purified by agarose gel electrophoresis. All DNA fragments were radiolabeled as described previously (7).

In situ hybridization with single cells

A previously described method (28) was adapted for the *in situ* hybridization as follows. After sedimentation and fixation of single cells onto pretreated microscopic glass slides, they were washed twice in PBS and prehybridized without further treatment in prehybridization solution (50% formamide, 0.6 M NaCl, 1 mM EDTA, 10 mM Tris – HCl, pH 7.6, 1 x Denhardt's solution, 500 μg/ml tRNA, and 150 μg/ml salmon sperm DNA). The hybridization was done in the same buffer with either 0.5 mM single strand radiolabeled λ_5 probe for 32 h at 35°C. The λ_5 probe had 6.4 x 10 d.p.m./nmol. The slides were washed within 24 h with several changes of 50% formamide, 0.6 M NaCl, 1 mM EDTA, 10 mM Tris – HCl, pH 7.6, and exposed to film emulsion as described (28). The slides were developed after exposure for 14 days. Grain counting was done under the microscope. A total of 400 cells were counted per population.

Northern blot analysis with oligonucleotides as probes

Two 32mer oligonucleotides, representing respectively sequences complementary to the 3′ untranslated region from position 928 to 959 of V_{preB1} and V_{preB2} (10) were synthesized by Dr H.-R. Kiefer at our Institute. They were labeled with [γ-^{32}P]ATP (Amersham, UK) and polynucleotide kinase (New England BioLab, USA). The filters were washed under low stringency with 0.3 x SSC, 0.1% SDS, at 50°C, and thereafter under high stringency with 0.1 x SSC, 0.1% SDS at 50°C and finally with 0.1% x SSC, 0.1% SDS at 60°C to remove the labeled probe from the filter. Prehybridization and hybridization were carried out with 900 mM NaCl, 90 mM Tris – HCl, pH 8.3, 6 mM EDTA, 1.0 x Denhardt's solution, 0.1% SDS, and 50 μg/ml heat denatured carrier DNA at 42°C.

Results

The expression of V_{preB} and λ_5 in progenitor HAFTL-1 cells, and in myeloid as well as pre B subclones derived from them

Total RNA was extracted from HAFTL-1 cells, and from the three myeloid sublines 2B7, Ig4, and 3g4 derived from HAFTL-1 cells and tested by Northern slot blot analyses for the presence of λ_5 transcripts (Fig. 1). Controls included the pre B cell clones NFS-5, NFS-25, ABLS19, and ABLS8, which all expressed λ_5, and the mature B cell BCL1 and the plasmacytoma ABPC69, which did not. Interestingly, the surface Ig-positive B lymphoma NFS-1 expressed low amounts of λ_5 (see later for expression of λ_5 in some surface Ig-positive B lineage cell lines). RNA from HAFTL-1 cells proved weakly positive, while RNA from the three myeloid sublines were negative within the limits of the assay.

Poly A$^+$ RNA purified from HAFTL-1 cells, from one myeloid subline (3G4) and from three pre B sublines cloned in the presence of LPS from HAFTL-1 cells (8) were then analyzed by

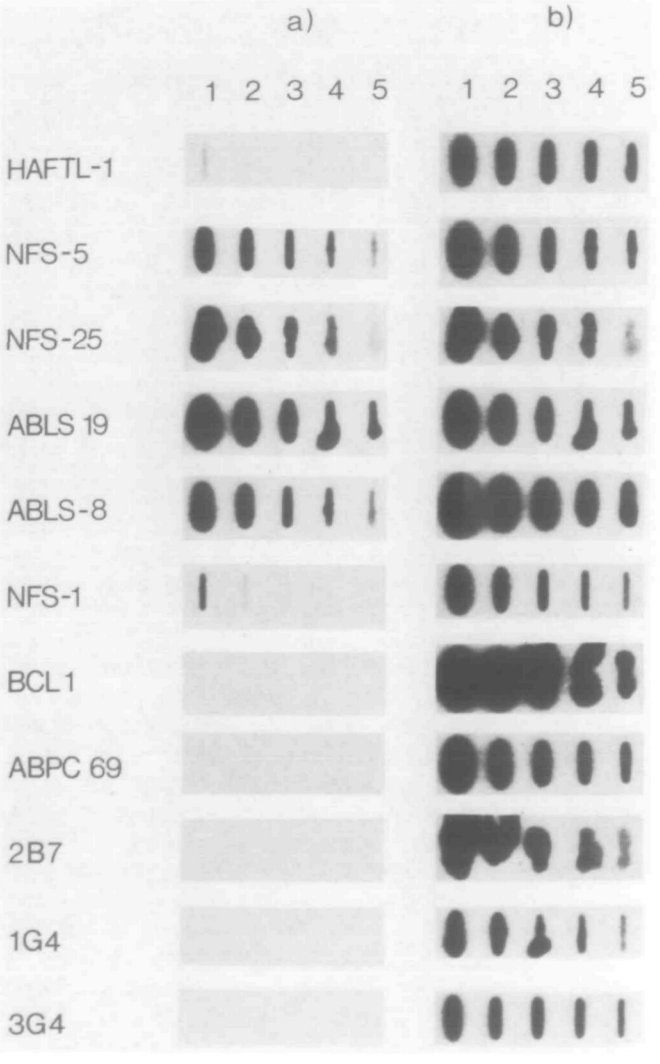

Fig. 1. RNA slot blot analysis of λ_5 transcripts in B lymphoid cell lines. Serially diluted RNA samples of various cell lines were blotted onto filter paper using Schleicher & Schuell manifold II. Poly A$^+$ RNA samples from pre B cell lines NFS-5 (Ly1$^+$), NFS-25 (Ly1$^+$), ABLS19 (Ly1$^+$), ABLS-8 (Ly1$^-$), mature B cell lines, NFS-1 (Ly1$^+$), BCL1 (Ly1$^+$), and plasmacytoma ABPC69 (Ly1$^+$) were blotted in two-fold dilutions starting from 10 μg. Total RNA samples from progenitor cells, HAFTL-1 (Ly1$^+$), and its myeloid subline, 2B7 (Ly1$^+$), IG4 (Ly1$^+$), and 3G4 (Ly1$^+$) were also blotted in 2-fold dilutions starting from 50 μg. The same filter was hybridized successively with λ_5 (a) and β-actin (b) probes.

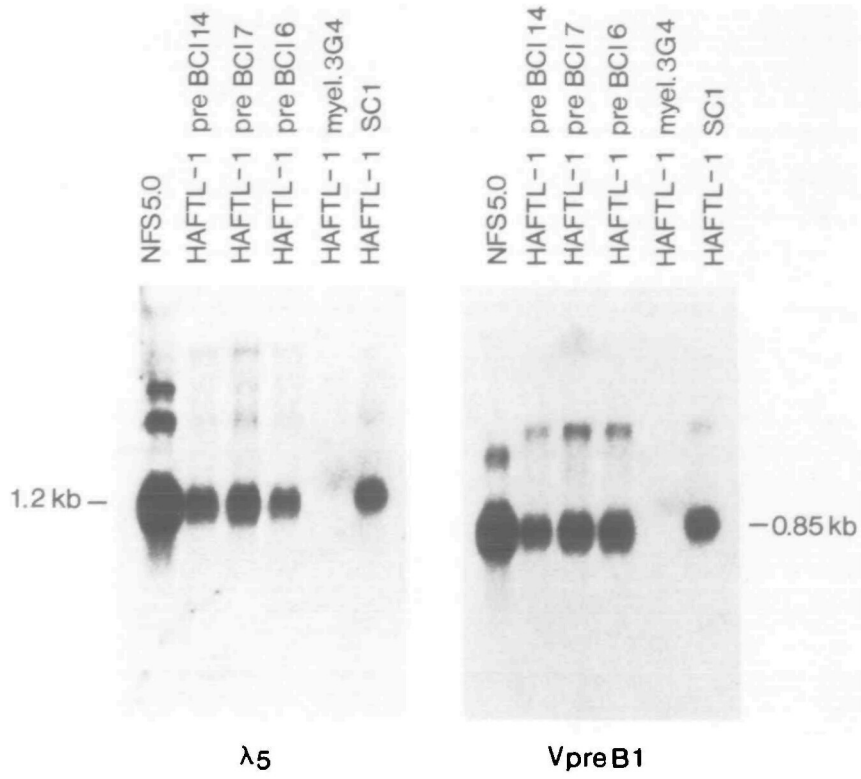

Fig. 2. Northern blot analysis of λ_5 and V_{preB1} transcripts in mouse HAFTL-1 cell lines. Poly A selected RNAs from various mouse HAFTL cell lines, the progenitor B cell line (HAFTL-1.Sc1), the pre B cell lines (HAFTL pre B Sc 6, 7, 14), the myeloid cell line (HAFTL-1 myel Sc 3G4), and from the mouse pre B cell line NFS-5 were extracted, electrophoresed, and transferred to a nitrocellulose filter. The filter was hybridized with oligolabeled pZ121 (V_{preB1}) or pZ183-1a (λ_5) as probes, washed with 0.1 × SSC, 0.1% SDS at 50°C, and exposed to X-ray film.

Northern blotting for the presence λ_5 as well as V_{preB} sequences. As a positive control the V_{preB}/λ_5-positive NFS-5 (see below) was included in this assay. Figure 2 shows that HAFTL-1, the three pre B subclones and NFS-5 were positive, while the myeloid subclone, within the limits of this assay, was negative. The sizes of λ_5 and V_{preB} were 1.2 and 0.85 kb respectively and, therefore, indistinguishable from the corresponding mRNAs found in other pre B cell lines (1 – 4). The higher molecular weight species of RNA also hybridizing with the λ_5 and V_{preB1} probe are thought to be precursor forms (32). We conclude from these experiments that the λ_5 and V_{preB} genes are not expressed in myeloid sublines of the V_{preB}/λ_5-positive HAFTL-1 cells.

The frequency of HAFTL-1 cells and their myeloid and pre B lymphoid subclones which express λ_5

Since the level of V_{preB} and λ_5 expression in the HAFTL-1 progenitors appeared somewhat lower than that observed in pre B cell lines (see Figs 1 and 2) it was conceivable that only a subpopulation of the HAFTL-1 cells expressed the two genes. This might be due to a subpopulation which might have already differentiated to pre B-type cells, while true progenitor cells would not express V_{preB}/λ_5. We therefore tested the expression of λ_5 in single cells in *in situ* hybridization. It has been estimated that the *in situ* hybridization method used here detects 15 – 25 specific mRNA molecules per cell (28) As shown in Fig. 3, in the three myeloid subclones, most cells did not express λ_5 at detectable levels. The major population of cells in three pre B cell subclones expressed λ_5, estimated by comparison with the *in situ* hybridization of cells with known specific mRNA expression at approximately the same intensities, to be at an average level of 15 – 20 specific mRNA molecules above background. In three progenitor lines, 50 – 80% of the cells were λ_5 positive. A homogenous distribution of silver grains per cell was found for the cell populations investigated, indicating that the majority of cells are, indeed, expressing λ_5, although at 1.5- to 2-fold lower levels then pre B lines.

Three conclusions can be drawn from these results. (i) HAFTL-1 progenitor cells, express V_{preB}/λ_5 *before* they differentiate to either myeloid or pre B cells. (ii) Differentiation along the myeloid, but not the pre B pathway of differentiation results in down-regulation of V_{preB}/λ_5 expression. (iii) Differentiation along the pre B pathway results in a 1.5- to 2-fold increase in λ_5 expression.

Expression of λ_5 in transformed pre B cell lines which express only H chain genes, or H chain plus L chain genes

Total cellular RNA extracted from the cell lines NFS-5.3 (expressing only μH chain RNA), NFS-5.4$\varkappa$ (expressing μH and $\varkappa$L chain RNA), NFS-5.4$\varkappa\lambda$ (expressing μH, $\varkappa$L, and λL chain RNA), and NFS-5.4 λ (expressing μH and λL chain mRNA) were analyzed on Northern blots with a λ_5 probe, and compared with control Northern blots detecting μH, $\varkappa$L, and λL chain RNA sequences. The results in Fig. 4 show that λ_5 is expressed at

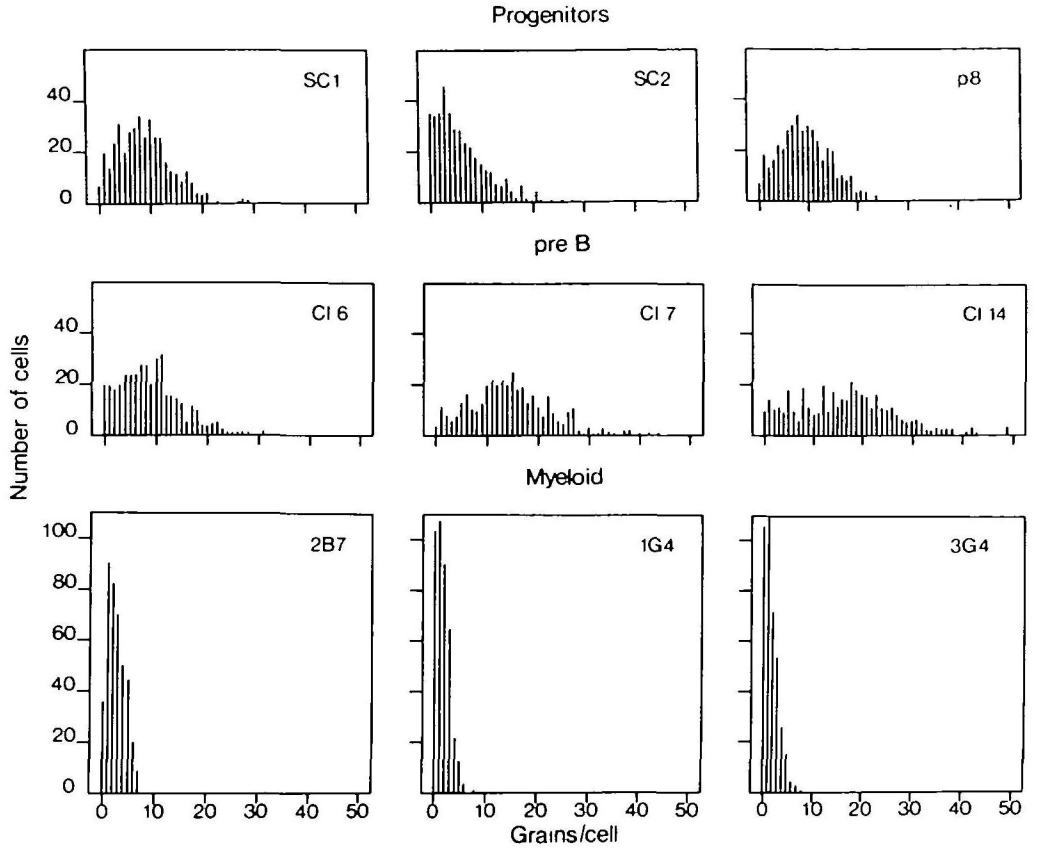

Fig. 3. Expression of λ_5 in single cells of three progenitor cell lines, three pre B subclones, and three myeloid subclones of the HAFTL-1 cell line. *In situ* hybridization of single cells on glass slides with a radiolabeled λ_5 probe, exposure to X-ray film emulsion, and counts of silver grains over single cells were done as described in Methods Exposure for 14 days. Background over area without cells contained less than 5 silver grains

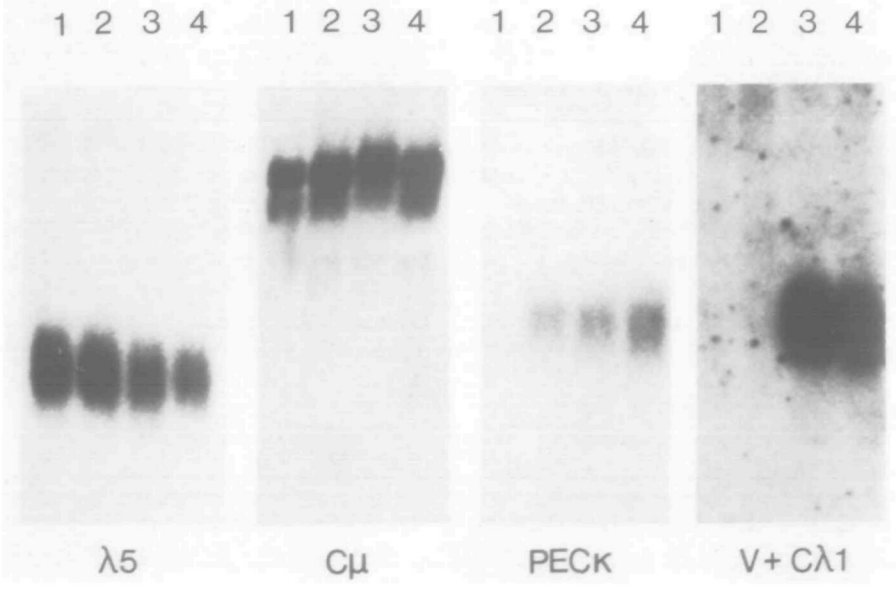

Fig. 4. λ_5 expression in the progressively Ig gene-rearranging NFS-5 sublines. Northern blot analysis with RNA samples from NFS-5 sublines cloned by FACS sorting for Ig phenotype as surface Ig-negative 5.3 (μ^+, κ^-) (1), surface κ^+ 5.4κ (μ^+, κ^+) (2), surface κ^+ and Ig λ^+ 5.4 $\kappa\lambda$ (μ^+, κ^+, λ^+) (3), and surface Ig λ^+ 5.4 λ (μ^+, κ^+, λ^+) (4). The filter was hybridized successively as in Fig. 1 with λ_5, μ, κ, and λ_1 specific probes.

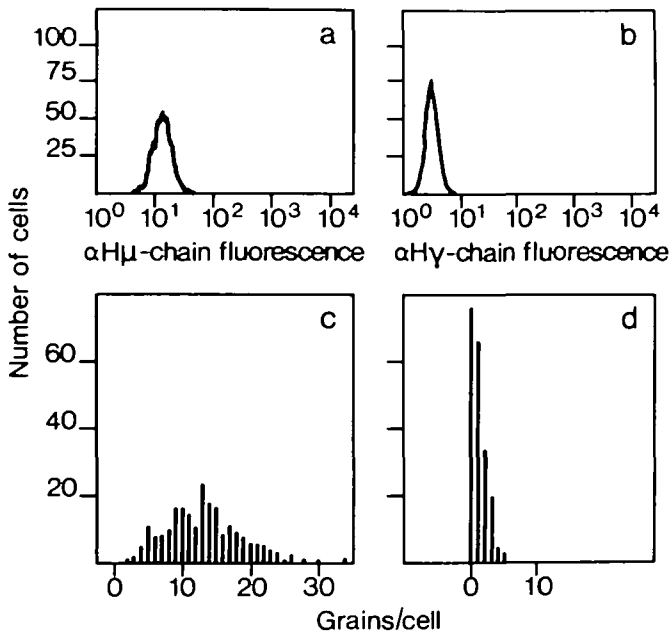

Fig. 5. Expression of surface Ig and intracellular λ_5-specific RNA in a 70Z/3 pre B lymphoma subline (70Z/3-PT) as determined with $H\mu$-chain specific antibodies (a) and $H\gamma$ specific antibodies (b) in FACS, and as probed by *in situ* hybridization of single cells with the 580 base long labeled λ_5 specific probe pZ183-1a (c = negative strand, d = positive strand). Exposure for 14 days. Background over area without cells contained less than 5 silver grains.

comparable levels in all cells, i.e. also in those expressing $\varkappa$L and λL chains. We conclude from these results that, at least in this transformed cell line of the B lineage, continued rearrangement of Ig genes and final expression of Ig on the surface do not turn off V_{preB} and λ_5 expression.

Induction of 70Z/3 pre B lymphoma cells by LPS is known to lead to the expression of the previously rearranged, through transcriptionally silent, $\varkappa$L chain locus (18,22). Data in Fig. 7 (lanes 10 and 11) show that LPS stimulation of 70Z/3 cells does not turn off λ_5 expression. The same result was found for V_{preB} expression in a separate experiment (data not shown).

This result further supports the conclusion that expression of surface Ig does not turn off λ_5 expression in transformed cell lines. This conclusion is also supported by the analysis of V_{preB}/λ_5 in a 70Z/3 pre B lymphoma subline which constitutively expresses Ig on its surface and which continues to express V_{preB}/λ_5 (Fig. 5). It is also of interest that the 38C-13 B lymphoma cell line expresses V_{preB}/λ_5 (Fig. 6). This cell line has previously been characterized as a cell type near the resting, mature B cell stage (16 – 18). The Ig6.11 clone expresses μ and $\varkappa$ chains on the surface (34). It also expresses a V_{preB}/λ_5 pseudo light chain complex that can be immunoprecipitated with an anti-μ body, but not with an anti-$\varkappa$ antibody (34). In the Ig6.11 line, the V_{preB} and λ_5 genes are expressed at a level comparable to Abelson transformed pre B cell line 38B9, while $\varkappa$ chain is also simultaneously expressed, although weakly compared with $\varkappa$ chain expression of the mature B cell line WEHI279 (Fig. 6).

The PAB clone 5 is a stroma and IL-7-dependent pre B clone which has DJ rearrangements at both H chain loci (35). It normally differentiates into surface Ig-positive cells when stroma or IL-7 is removed from the culture. A surface $\mu\varkappa$ positive variant cell line accidentally established from the original clone has kept the IL-7 dependency and lost the stromal dependency (Rolink *et al.*, unpublished data). The data in Fig. 6 shows that this surface Ig-positive, IL-7-dependent variant cell line continues to express V_{preB}/λ_5.

The Abelson transformed pre B cell line P-8, with a phenotype of $\mu^+\varkappa^-$, is a subline of the 300-19 parent line (31). Two sublines, B1P87-2-2 ($\mu^+,\varkappa^-,\lambda^+$) and B3P816-1-11-18 ($\mu^+\varkappa^+$) were generated by subcloning from the original Abelson transformed P-8 cell line. Data in Fig. 6 show that the V_{preB} and λ_5 genes are constitutively expressed in all these sublines, i.e. also in cells which are surface Ig-positive either with $\varkappa$ or λ L chains These results support the conclusion that expression of surface Ig does not by itself turn off V_{preB}/λ_5 expression (36).

Expression of λ_5 in pre B hybridomas

Fusions of normal pre B lymphocytes with the azaguanine resistant plasmacytoma cell line X63-Ag8.653 leads to pre B hybridomas, in which the status of Ig rearrangements typical of pre B cells, i.e. with rearranged H chain genes, but germline L chain genes, has been preserved. We have tested five such pre B hybridomas. The results, shown in Fig. 7, indicate that λ_5 is turned off in all five pre B hybridomas. All five hybridomas were shown to continue to carry V_{preB} and λ_5 genes. This indicates that the phenotype of the mature B cell dominates over the pre B phenotype in the expression of V_{preB}/λ_5 and suggests that *trans*-acting factors expressed in mature B cells and plasma cells might turn off the expression of the V_{preB}/λ_5 locus in these hybridomas.

In summary, these results indicate that expression of λ_5 in transformed cell lines and tumors is not regulated by the expression of H, $\varkappa$L, or λL chains, LPS reactivity or the Ly1 surface antigen, but appears to be down-regulated in myeloid cells, mature B cells and plasma cells, possibly by *trans*-acting factors.

Distinction of V_{preB1} from V_{preB2} expression

Since V_{preB1} and V_{preB2} sequences show 97% sequence identity in their two exons, the intervening intron, and the 5′ and 3′ flanking regions, expression at the RNA level monitored with the probes described above could not distinguish between the two genes. In order to distinguish between expression of V_{preB1} and V_{preB2}, two 32mer oligonucleotides were synthesized which differ internally by three nucleotides, and which represent the negative strands of the corresponding 3′ untranslated regions of V_{preB1} and V_{preB2}. While under mild stringencies of washing conditions, both oligonucleotides could be expected to hybridize with both V_{preB1} and V_{preB2} derived RNA, high stringency washing-conditions should distinguish between the two. Northern blots of poly A-selected nuclear RNA from 18-81 and 70Z/3 pre B cells, from the V_{preB}/λ_5-expressing 38C13 cells, and from the A32-26 T hybridoma cells were hybridized under low and high stringency washing conditions with the V_{preB1}- as well as the V_{preB2}-specific 32mer oligonucleotides.

It is evident from the results in Fig. 8 that, under low stringency, both oligonucleotides detected V_{preB} expression in 18-81, 70Z/3, and 38C-13 cells, but not in A32-26 cells. Under low stringency, 2.0 and 0.85 kb RNA species could be detected with the V_{preB1} oligo probe. Under high stringency, the V_{preB2}-specific

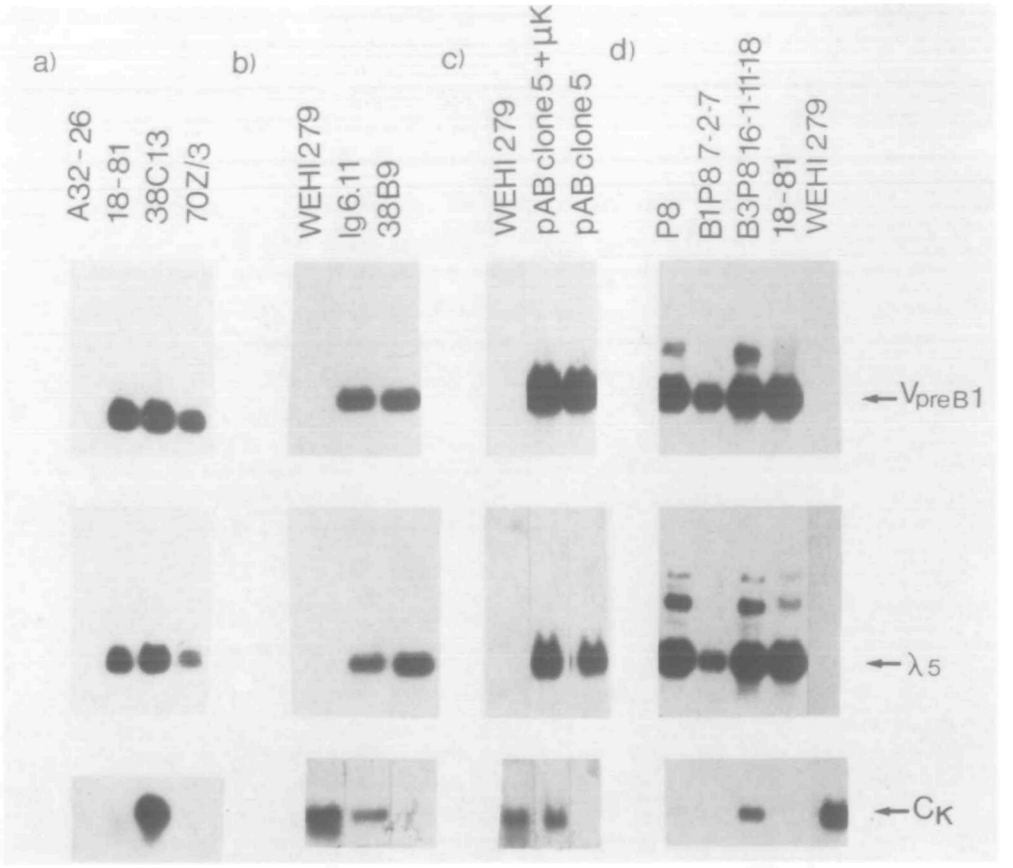

Fig. 6. Expression of V_{preB}/λ_5 transcripts in intermediate B cells Northern blot analysis with RNA samples; RNAs of intermediate cells from pre B to B, 38C13 (a), Ig6 11 (b), and PAB clone 5 + μx (c), were extracted and 10 μg of total RNAs was transferred on a nylon membrane and hybridized RNAs from a mouse T cell hybridoma A32-26 (a), mouse pre B cells 18-81 (a, d), and 70Z/3 (a), 38B9 (b), PAB clone 5 (c) a mouse mature B cell line WEHI279 are used as controls Poly A selected 1 μg RNA from the Abelson transformed pre B cell line (P8 μ^+x^-) and Abelson transformed intermediate cell lines (B1P87-2-7; $\mu^+x^-\lambda^+$; B3P816-11-18; μ^+x^+) were loaded onto the gel and transferred

oligonucleotides continued to hybridize to both the 2.0 and the 0.85 kb RNA, while the V_{preB1}-specific oligonucleotide detected 0.85 kb mature mRNA, but not the 2.0 kb transcript. The 9.1 kb precursor spanning the V_{preB1}/λ_5 locus is detected by the V_{preB1} probe (32).

Interestingly, high stringency hybridization with the V_{preB2}-specific oligonucleotide reduced the signal in the 0.85 kb mature mRNA detected with RNA from the 70Z/3 pre B cell line. It suggests that 70Z/3 cells express much less V_{preB2} or only V_{preB1}. It also shows that V_{preB1} is, in fact, expressed as mature 0.85 kb mRNA.

Discussion

Different stages in the development of B lymphocytes are distinguished by the expression of specific surface markers (4 – 6) and by the status of rearrangement of the H and L chain genes (1,2). Pre B lymphocytes have been defined as cells in which rearrangements of the H and L chain genes are ongoing. They have also been characterized as cells which are not yet reactive to antigens or mitogens (3). Once pre B cells have completed their H and L chain gene rearrangements and have expressed Ig on the surface membrane, they are thought to fall into a resting state, in which they become reactive to antigens and mitogens.In this transition to the resting state a cell has been defined as pre B which is smaller than earlier pre B cells, i.e. is on its way to the resting state, is surface Ig-positive, but is not yet a mature B cell and, therefore, could suffice the definition of a tolerizable B cell (30).

On the other side of the spectrum of precursor cells of the B lineage, i.e. at the points of differentiation of stem cells into cells of the different hemopoietic lineages and, in particular, into the B lineage before rearrangements of the H chain genes, little is known of the hierarchies of cellular development characterized by surface markers and reactivities to growth factors. Expression of the V_{preB}/λ_5 locus has been found to be restricted to normal B lineage cells and to their transformed and malignant counterparts. Expression of this locus, at 50 – 100 copies per cell, can thus be regarded as yet another marker of the pre B stage of B cell development. So far, the earliest cells in which V_{preB} and λ_5 have been found to be expressed are those in which both chromosomes have already rearranged D_H with J_H. In the present study we use V_{preB} and λ_5 expression to find earlier stages of cells committed to the B lineage before, or at the

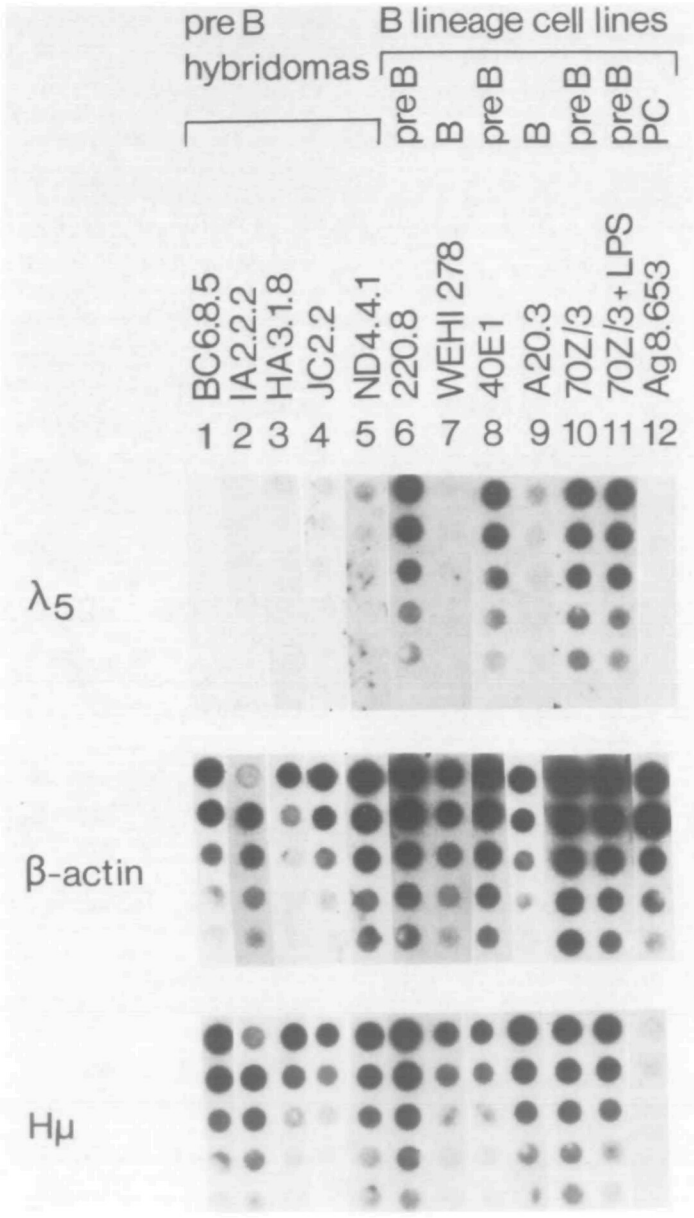

Fig. 7. Quantitative Northern dot blot analysis of λ_5 transcripts in cell lines. Serial 2-fold dilutions were blotted on nitrocellulose filters for hybridization with the labeled λ_5 probe (see Methods) Filters were first probed with the λ_5 probe, then washed, probed with a β-actin probe, washed again, and finally probed with a Hμ probe. The highest concentration of RNA contained between 1 and 5 μg total RNA per dot. Lanes 1 – 5 contain RNA from five pre B hybridomas, lane 12 RNA from the parental Ag8.653 cell line used in the fusions Lanes 10 and 11 contain RNA from 70Z/3 B lymphoma cells, either not stimulated (lane 10) or stimulated with 50 μg/ml LPS for 2 days (lane 11). Lanes 6 and 8 contain RNA from pre B lymphoma lines 220.8 and 40E-1, lanes 7 and 9 from mature B cell lines, WEHI 278, and A20.3.

beginning, of the Ig gene rearrangement processes, and to define more clearly the state of transition from a precursor B cell to a mature B cell. In these studies, we assume that the transformed and malignant cell lines are representatives of normal cells which faithfully express the V_{preB}/λ_5 locus as do their normal counterparts.

Our findings expand previous observations on the pre B related expression of the V_{preB} and λ_5 genes and show that they are expressed in both the Ly1$^+$ and Ly1$^-$ lineage B cells and indicate that they might be expressed in progenitors before the decision point to become either myeloid or pre B type, and maybe even before or at the point when Ig H chain gene segments begin to be rearranged. Since V_{preB} and λ_5 are also expressed in early progenitors their expression cannot be directly regulated in association with complete rearrangements of the H chain locus.

At the more differentiated stage of B cells when V_{preB} and λ_5 are about to be turned off, several surface Ig-positive B lineage

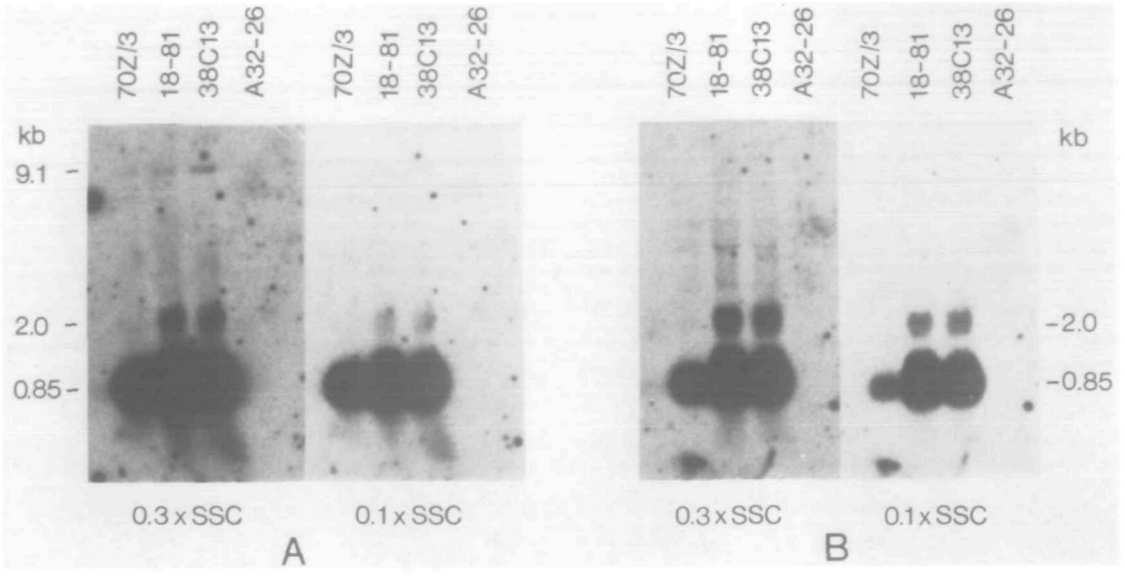

Fig. 8. Northern blot analysis to distinguish V_{preB1} from V_{preB2} transcription. Poly(A)-selected nuclear RNA (10 μg) from 70Z/3, 18-81, and 38C13 mouse pre-B cell lines, and from the A32-26 mouse T cell hybridoma was separated on 1.0% agarose gel and transferred to a nitrocellulose filter. The filters were hybridized with end-labeled 32mer oligonucleotides specific for V_{preB1} (A) or V_{preB2} (B) genes, washed first with 0.3 x SSC, 0.1% SDS at 50°C, then with 0 1 x SSC, 0.1% SDS at 50°C, and exposed to X-ray film (Kodak XS-1) after each wash step. For further details see Methods and the text

lines have been identified which continue to express V_{preB}/λ_5, suggesting that expression of κL or λL chains does not directly down-regulate V_{preB} and/or λ_5 expression. The results obtained with the different NFS-5 subclones differing in L chain expression have also been observed with another cell line, 300-19 and PAB clone 5, which continues to rearrange Ig H and L chain genes and, at the same time, continues to express V_{preB}/λ_5. These findings also offer a possible explanation for the origin and development of the surface Ig-positive B lineage cell lines 38C-13, Ig6.11, and NFS-1, and for surface Ig-positive human B cell lines expressing human counterparts of V_{preB} and λ_5 (37). Recently, Cherayil and Pillai detected a subpopulation of bone marrow B cells expressing the Ig H chain and λ_5 on the surface (36,38), that might be an original source of tumor cell lines. These cell lines could have originated from an L chain-negative pre B cell, and might have continued, as do the NFS-5, 300-19, and 70Z/3 cells lines, their molecular program of rearrangements of Ig genes, ending in a surface Ig-positive, i.e. L chain expression cell. While successive Ig gene rearrangements have been used to characterize different stages of B cell development, our results argue, at least for transformed cells, that these rearrangements must not necessarily signal the cell to continue other programs of differentiation, such as the change of expression of V_{preB}/λ_5. However, all this does is not rule out a more complex involvement of any of these markers in V_{preB}/λ_5 expression in which the regulation might depend on the expression of either one of these markers together with other, yet unknown, gene products.

The observation that pre B hybridomas do not express V_{preB} and λ_5 suggests that their expression is negatively regulated in mature B cells and in plasma cells. Since expression is also turned off in myeloid cells it could be that the production of *trans*-acting factors might down regulate expression in both mature B cells and myeloid cells.

Altogether the pattern of expression of V_{preB}/λ_5 in normal and transformed cells suggests that the locus should be positively up-regulated in pre B cells, possibly by tissue specific promoters and enhancers, and down-regulated tissue-specifically at least in myeloid cells, mature B cells, and plasma cells. The genetic *cis*-elements and their corresponding *trans*-acting factors are now under investigation (39,40).

Acknowledgements

We thank Ms Denise Richterich, Heidi Bachtold, and Mr Wyn Davies for able technical assistance. We thank Drs James Kaufman and Yair Argon for critical reading of our manuscript. Part of this work was supported by The Basel Institute for Immunology, founded and supported by F. Hoffmann-La Roche & Co. Ltd, Basel, Switzerland, and NIH grants AI 18742, AI 30879, and CA 13148.

Abbreviations

FACS	fluorescence activated cell sorter
H	heavy
IMDM	Iscove's modified Dulbecco's medium
L	light
LPS	lipopolysaccharide
pre B	precursor cells
pro B	progenitor cells

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